

IN THE CLAIMS:

This listing of claims will replace all prior versions, and listings, of claims in the application:

1. (Currently Amended) A method of quantitation of ~~an~~ amount of a glycated form of a selected protein ~~which is glycated~~ relative to the total amount of the selected protein (non-glycated and glycated forms) in a biological sample which comprises:

(aA) contacting a solid support matrix which comprises a negatively charged group and a hydroxyboryl compound and which has a measurement area, with an aliquot of biological sample sufficient to cover said measurement area;

(bB) contacting said solid support matrix with an aliquot of a first buffer wherein (i) said first buffer has a pH selected to allow both glycated and non-glycated forms of the selected protein to bind to said solid support matrix and where both glycated and non-glycated forms of the selected protein bind to the solid support matrix and wherein (ii) said first buffer is applied in an amount sufficient to rinse off protein which does not bind to the solid support matrix;

(cC) quantitating amount of the selected protein bound to said measurement area using measurement of a selected property of ~~said the selected~~ protein to give a first bound protein reading to determine amount of total bound selected protein;

(dD) contacting said solid support matrix with an aliquot of a second buffer wherein (i) said second buffer has a pH selected to allow the glycated form of the selected protein to bind to said solid support matrix and where the glycated form of the selected protein binds to the solid support matrix but where the non-glycated form of the selected protein does not substantially bind to said solid support matrix and wherein (ii) said second buffer is applied in an amount sufficient to rinse off the non-glycated protein;

(eE) quantitating amount of the selected protein bound to said measurement area using measurement of ~~the a selected property measured in step (c)~~ of the selected protein to give a second bound protein reading to determine amount of glycated form of the selected protein; and

(fF) calculating ratio of relative amount of the glyated form of the selected protein to amount of total bound selected protein using said first and second bound protein readings.

2. (Previously Presented) A method according to claim 1 wherein the property is measured using an optical reading.

3. (Original) A method according to claim 2 wherein the optical reading is absorbance or reflectance at a specified wavelength.

4. (Currently Amended) A method according to claim 3 wherein the selected protein is hemoglobin ~~the glyated protein is glyated hemoglobin.~~

5. (Currently Amended) A method according to claim 3 wherein the selected protein is albumin ~~the glyated protein is glyated albumin.~~

6. (Currently Amended) A method for quantitation of ~~an~~ amount of a glyated form of a selected protein ~~which is glyated~~ relative to the total amount of the selected protein (non-glyated and glyated forms) in a biological sample which comprises:

(aA) contacting a solid support matrix which comprises a negatively charged group and a hydroxyboryl compound and which has a measurement area with an aliquot of a biological sample sufficient to cover said measurement area;

(bB) contacting said solid support matrix with an aliquot of a first buffer, wherein (i) said first buffer has a pH of about 5.0 to about 7.0 which allows both glyated and non-glyated forms of the selected protein to bind to the solid support matrix and where both glyated and non-glyated forms of the selected protein bind to the solid support matrix and wherein (ii) said first buffer is applied in an amount sufficient to rinse off protein which does not bind to the solid support matrix at a pH of about 5.0 to about 7.0;

(cC) quantitating amount of the selected protein bound to said measurement area using measurement of a selected property of the selected protein to give a first bound protein reading to determine amount of total bound selected protein;

(dD) contacting said solid support matrix with an aliquot of second buffer, wherein (i) said second buffer has a pH of about 8.0 to about 10.0 (a) which allows the glyated

form of the selected protein to bind to the solid support matrix and where the glycated form of the selected protein binds to the solid support matrix but (b) which does not allow the non-glycated form of the selected protein to substantially bind to said solid support matrix and wherein (ii) said second buffer is applied in an amount sufficient to rinse off non-glycated protein which does not bind to the solid support matrix at a pH of about 8.0 to about 10.0;

(eE) quantitating amount of the selected protein bound to said measurement area using measurement of a selected property of the selected protein to give a second bound protein reading to determine amount of glycated form of the selected protein; and

(fF) calculating relative ratio of amount of the glycated form of the selected protein to amount of total bound selected protein in said sample using said first ~~bound protein reading~~ and said second bound protein ~~reading readings~~.

7. (Previously Presented) A method according to claim 6 wherein said first and second bound protein readings measure the same selected property of the protein.

8. (Previously Presented) A method according to claim 7 wherein the selected property is measured using an optical reading.

9. (Original) A method according to claim 8 wherein the optical reading is absorbance or reflectance at a specified wavelength.

10. (Currently Amended) A method according to claim 9 wherein the selected protein is hemoglobin ~~the glycated protein is glycated hemoglobin.~~

11. (Currently Amended) A method according to claim 9 wherein the selected protein is albumin ~~the glycated protein is glycated albumin.~~

12. (Currently Amended) A method of quantitation of ~~an~~ amount of a glycated form of hemoglobin ~~which is glycated~~ relative to total amount of hemoglobin (non-glycated and glycated forms) in a biological sample which comprises;

(aA) adding said sample to a sample application site which is in communication with a solid support matrix, ~~which~~ wherein the solid support matrix comprises a negatively charged group and a dihydroxyboryl compound and ~~which~~ has a measurement area;

(bB) adding an aliquot of a first buffer at said sample application site, wherein (i) said first buffer has a pH between about 5.0 and about 7.0 which allows the glycated and non-glycated forms of hemoglobin to bind to the solid support matrix and where both glycated and non-glycated hemoglobin bind to the solid support matrix and wherein (ii) the first buffer is applied in an amount sufficient to rinse off hemoglobin which does not bind to the solid support matrix;

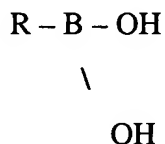
(eC) making a first optical reading of said measurement area at a wavelength at which hemoglobin absorbs light to determine amount of total bound hemoglobin;

(dD) adding an aliquot of a second buffer at said sample application site, wherein (i) said second buffer has a pH between about 8.0 and about 10.0 (a) which allows the glycated form of hemoglobin to bind to the solid support matrix and where the glycated form of hemoglobin binds to the solid support matrix but (b) which does not allow the non-glycated form of hemoglobin to substantially bind to the solid support matrix and wherein (ii) said second buffer is applied in an amount sufficient to rinse off the non-glycated form of hemoglobin;

(eE) making a second optical reading of said measurement area at a wavelength at which hemoglobin absorbs light to determine amount of glycated form of hemoglobin; and

(fF) calculating relative ratio of amount of the glycated form of hemoglobin to amount of total bound hemoglobin in said blood sample using said first and second optical readings.

13. (Original) A method according to claim 12 wherein said dihydroxyboryl compound has the formula



wherein R is selected from the group consisting of phenyl, substituted hydrogen, and alkyl of 1 to about 6 carbon atoms.

14. (Original) A method according to claim 13 wherein R is selected from the group consisting of phenyl, m-aminophenyl, hydrogen, ethyl, 1-propyl and 2-methyl-1-butyl.

15. (Original) A method according to claim 14 wherein R is m-aminophenyl.

16. (Original) A method according to claim 13 wherein the negatively charged group is selected from the group consisting of carboxylate, sulfate, sulfonate, sulfinate and phosphate.

17. (Original) A method according to claim 16 wherein the negatively charged group is carboxylate.

18. (Original) A method according to claim 12 wherein said solid support matrix is selected from the group consisting of cellulose, nitrocellulose, cellulose acetate, polyacrylamide, agarose polyacrylamide copolymer, agarose, starch, nylon, nylon polyesters, dextran, cross-linked dextran, dextran acrylamide copolymer, cross-linked hydroxyethylmethacrylate substituted cross-linked polystyrenes, and polyvinylalcohol.

19. (Original) A method according to claim 18 wherein said solid support matrix is carboxy cellulose.

20. (Original) A method according to claim 12 wherein said first buffer is selected from the group consisting of MES, MOPS and HEPES.

21. (Original) A method according to claim 12 wherein said second buffer is ammonium acetate or taurine.

22. (Withdrawn - Currently Amended) A method of quantitation of ~~an~~ amount of a glycated form of a selected non-hemoglobin protein ~~which is glycated~~ relative to total amount of the selected protein (non-glycated and glycated forms) in a biological sample wherein said selected protein is optionally labeled with a ~~protein~~ specific selected protein labeling agent which comprises:

(aA) adding said sample to a sample application site which is in communication with a solid support matrix, ~~which~~ wherein the solid support matrix comprises a negatively charged group and a dihydroxyboryl compound and ~~which~~ has a measurement area;

(bB) adding an aliquot of a first buffer to said sample application site, wherein (i) said first buffer has a pH between about 5.0 and about 7.0 which allows glycated and non-glycated forms of the selected protein to bind to the solid support matrix and where both glycated and non-glycated forms of the selected protein bind to the solid support matrix and wherein (ii)

the first buffer is applied in an amount sufficient to rinse off protein which does not bind to the solid support matrix;

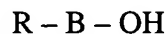
(eC) making a first optical reading of said measurement area at a wavelength at which said ~~non-hemoglobin~~ selected protein or said specific selected protein labeling agent absorbs light to determine amount of total bound selected non-hemoglobin protein;

(dD) adding an aliquot of a second buffer to said sample application site wherein (i) said second buffer has a pH between about 8.0 and about 10.0 (a) which allows the glycated form of the selected protein to bind to the solid support matrix and where the glycated form of the selected protein binds to the solid support matrix but (b) which does not allow the non-glycated form of the selected non-hemoglobin protein to substantially bind to the solid support matrix and wherein (ii) the second buffer is applied in an amount sufficient to rinse off non-glycated protein;

(eE) making a second optical reading of said measurement area at a wavelength at which said ~~non-hemoglobin~~ selected protein or said specific selected protein labeling agent absorbs light to determine amount of glycated non-hemoglobin protein; and

(fF) calculating relative ratio of amount of the glycated form of the selected non-hemoglobin protein to amount total of bound selected non-hemoglobin protein in said sample using said first and second optical readings.

23. (Withdrawn - Original) A method according to claim 22 wherein said dihydroxyboryl compound has the formula:



wherein R is selected from the group consisting of phenyl, substituted phenyl, hydrogen, and alkyl of 1 to about 6 carbon atoms.

24. (Withdrawn - Original) A method according to claim 23 wherein R is selected from the group consisting of phenyl, m-amino phenyl, hydrogen, ethyl, 1-propyl and 2-methyl-1-butyl.

25. (Withdrawn - Original) A method according to claim 23 wherein R is m-aminophenyl.
26. (Withdrawn - Previously Presented) A method according to claim 22 wherein the negatively charged group is selected from the group consisting of carboxylate, sulfate, sulfonate, sulfinate and phosphate.
27. (Withdrawn - Original) A method according to claim 26 wherein the negatively charged group is carboxylate.
28. (Withdrawn - Original) A method according to claim 22 wherein said solid support matrix is selected from the group consisting of cellulose, nitrocellulose, cellulose acetate, polyacrylamide, agarose polyacrylamide copolymer, agarose, starch, nylon, nylon polyesters, dextran, cross-linked dextran, dextran acrylamide copolymer, cross-linked hydroxyethylmethacrylate substituted cross-linked polystyrenes, and polyvinylalcohol.
29. (Withdrawn - Original) A method according to claim 28 wherein said solid support matrix is carboxy cellulose.
30. (Withdrawn - Original) A method according to claim 22 wherein said first buffer is selected from the group consisting of MES, MOPS and HEPES.
31. (Withdrawn - Original) A method according to claim 22 wherein said second buffer is ammonium acetate or taurine.
32. (Withdrawn - Currently Amended) A method according to claim 22 wherein said sample is labeled with a specific selected protein labeling agent.
33. (Withdrawn - Original) A method according to claim 22 wherein said sample is serum or plasma.
34. (Withdrawn - Currently Amended) A method according to claim 33 wherein ~~said~~ the non-hemoglobin protein is albumin ~~non-hemoglobin glyated protein is glyated albumin.~~

Claims 35 to 40 (cancelled).